Title: Extracellular vesicles secretion by *Mycoplasma mycoides* subsp. *mycoides,* the etiological agent of contagious bovine pleuropneumonia

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Extracellular vesicles (EV) are nano-sized, membrane-derived, non-self-replicating, spherical structures shed into the extracellular environment by both eukaryotic and prokaryotic cells. In bacteria, EV carry virulence factors like adhesins, toxins and immunomodulatory molecules. They are of importance for the bacteria –host interplay and hence vaccine development. Within the *Mycoplasma (M.)* genus EV have only been described, briefly, in *M. gallisepticum* so far. The aim of this study was to investigate the potential secretion of EV by *M. mycoides* subsp. *mycoides (Mmm)*, the etiological agent of contagious bovine pleuropneumonia, and to characterize their membrane protein content.

Several culture conditions were tested to produce EV of *Mmm* strain Afadé *in vitro*. Vesicles were collected using a classical ultracentrifugation method and observed by

transmission electron microscopy. Different approaches, such as gradient density and cultures in minimal medium were tested in order to improve EV purification. EV membrane proteins were extracted with triton x-114 and identified by mass spectrometry.

Mmm was shown to release EV *in vitro*, their size and concentration being dependent on growth conditions. *Mmm* vesicles were produced by living cells and not by cells with decreased viability after stresses. Their release happened in a budding-way from the cell surface as observed by electron miscroscopy. Several virulence factors were identified in EV membranes suggesting that EV could be involved in host-mycoplasma interactions.

Mmm can produce *in vitro* extracellular vesicles that carry virulence factors. Whether this also happens *in vivo* has yet to be demonstrated.